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NEWPORT

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1. Your reference — 100203 GB -1

2. Patent application number *(The Patent Office will fill in this part)* — 0025859.0

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca AB
S-151 85
Sodertalje
Sweden

7522465003

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — SWEDEN

4. Title of the invention — CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)* — Allen Giles

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

AstraZeneca UK Ltd
Global Intellectual Property - Patents
PO BOX 272, Mereside, Alderley Park
Macclesfield, Cheshire, SK10 4GR

7522471002

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.

CHEMICAL COMPOUNDS

This invention relates to polymorphisms in the human $P2X_7$ gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the $P2X_7$ gene, and to the use of $P2X_7$ polymorphism in treatment of diseases with $P2X_7$ drugs.

The $P2X_7$ receptor (previously known as P2Z receptor), which is a ligand-gated ion channel, is present on a variety of cell types, largely those known to be involved in the inflammatory/immune process, specifically, macrophages, mast cells and lymphocytes (T and B). Activation of the $P2X_7$ receptor by extracellular nucleotides, in particular adenosine triphosphate, leads to the release of interleukin-1β (IL-1β) and giant cell formation (macrophages/microglial cells), degranulation (mast cells) and L-selectin shedding (lymphocytes). $P2X_7$ receptors are also located on antigen-presenting cells (APC), keratinocytes, salivary acinar cells (parotid cells) and hepatocytes. Compounds acting at the $P2X_7$ receptor are therefore indicated as pharmaceuticals for use in the treatment of rheumatoid arthritis, osteoarthritis, psoriasis, allergic dermatitis, asthma, chronic obstructive pulmonary disease (COPD), hyperresponsiveness of the airway, septic shock, glomerulonephritis, irritable bowel disease, Crohn's disease, ulcerative colitis, atherosclerosis, growth and metastases of malignant cells, myoblastic leukaemia, diabetes, Alzheimer's disease, meningitis, osteoporosis, burn injury, ischaemic heart disease, stroke and varicose veins. For further background, the reader is referred to the following articles: North and Barnard in Current Opinion in Neurobiology 1997, **7**, 346-357; Rassendren, JBC, 1997, **273**, 5482-6; and Buell, Receptors and Channels, 1998, **5**, 347-354.

All positions herein of polymorphisms in the 5' UTR region of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 1 unless stated otherwise or apparent from the context.

All positions herein of polymorphisms in the exon regions of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 2 unless stated otherwise or apparent from the context.

All positions herein of polymorphisms in the intron regions of the $P2X_7$ polynucleotide relate to the position in SEQ ID NO 3 unless stated otherwise or apparent from the context.

positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3;

positions 76 ,155, 245, 270, 275, 348, 357, 430, 433, 460, 490 and 496 in the P2X7 polypeptide as defined by the position in SEQ ID NO: 4.

The term human includes both a human having or suspected of having a $P2X_7$ mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

The term polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene and corresponding alterations in expressed protein.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1 is any one of the following:

at position 936 is presence of C and/or A; at position 1012 is presence of T and/or C; at position 1147 is presence of A and/or G; at position 1343 is presence of G and/or A; and at position 1476 is presence of A and/or G.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the coding region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 2 is any one of the following:

at position 253 is presence of T and/or C; at position 488 is presence of G and/or A; at position 489 is presence of C and/or T; at position 760 is presence of T and/or G; at position 835 is presence of G and/or A; at position 853 is presence of G and/or A; at position 1068 is presence of G and/or A; at position 1096 is presence of C and/or G; at position 1315 is presence of C and/or G; at position 1324 is presence of C and/or T; at position 1405 is presence of A and/or G; at position 1448 is presence of C and/or T; at position 1494 is presence of A and/or G; at position 1513 is presence of A and/or C; at position 1628 is presence of G and/or T; and at position 1772 is presence of G and/or A.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the polymorphism in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3. is any one of the following:

Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
ELISA	Enzyme Linked ImmunoSorbent Assay
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

5

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Immunoassay techniques are known in the art e.g. A Practical Guide to ELISA by D M Kemeny, Pergamon Press 1991; Principles and Practice of Immunoassay, 2nd edition, C P Price & D J Newman, 1997, published by Stockton Press in USA & Canada and by Macmillan Reference in the United Kingdom.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of a drug acting at $P2X_7$.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the $P2X_7$ gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by $P2X_7$. This may be particularly relevant in the development of hyperlipoproteinemia and cardiovascular disease and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the $P2X_7$ gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.

According to another aspect of the present invention there is provided a human $P2X_7$ gene or its complementary strand comprising a variant allelic polymorphism at one or more of

intron E	4780 C→T 4845 C→T 4849 A→C
intron F	5021 T→C 5554 (GTTT)n=3,4 5579 G→C 5535 A→T
intron G	5845 C→T 6911 T→C

According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human $P2X_7$ gene and comprising an allelic variant selected from any one of the following:

Region	Variant SEQ ID NO: 1
5'UTR	936 A 1012 C 1147 G 1343 A 1476 G

Region	Variant SEQ ID NO: 2
exon 2	253 C
exon 5	488 A 489 T
exon 7	760 G
exon 8	835 A 853 A
exon 11	1068 A 1096 G
exon 12	1315 G
exon 13	1324 T 1405 G 1448 T 1494 G 1513 C 1628 T 1772 A

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism, preferably at one or more of the positions defined herein.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism at one of the positions defined herein.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

In another aspect of the invention, the polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphisms of relatively high frequency. The $P2X_7$ gene is on chromosome 12q24 (Buell et al, Receptors and Channels, 1998, 5,347-354). Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked

positions 4780, 4845, 4849, 5021, 5554, 5579, 5535, 5845 and 6911 in the intron region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 3; and

positions 76 ,155, 245, 270, 275, 348, 357, 430, 433, 460, 490 and 496 in the $P2X_7$ polypeptide as defined by the position in SEQ ID NO: 4;

and determining the status of the human by reference to polymorphism in $P2X_7$; and

ii) administering an effective amount of the drug.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs. The term "drug acting at $P2X_7$" means that drug binding with $P2X_7$ in humans is an important part of a drug exerting its pharmceutical effect in man.

According to another aspect of the present invention there is provided use of a drug acting at $P2X_7$ in preparation of a medicament for treating a disease in a human diagnosed as having a polymorphism therein, preferably at one or more of the positions defined herein.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising $P2X_7$ drug and instructions for administration of the drug to humans diagnostically tested for a polymorphism therein, preferably at one or more of the positions defined herein.

According to another aspect of the present invention there is provided an allelic variant of human $P2X_7$ polypeptide comprising at least one of the following:

a alanine at position 76 of SEQ ID NO 4;

a tyrosine at position 155 of SEQ ID NO 4;

a glycine at position 245 of SEQ ID NO 4;

a histidine at position 270 of SEQ ID NO 4;

a histidine at position 275 of SEQ ID NO 4;

a tyrosine at position 348 of SEQ ID NO 4;

a serine at position 357 of SEQ ID NO 4;

a arginine at position 430 of SEQ ID NO 4;

a valine at position 433 of SEQ ID NO 4;

a arginine at position 460 of SEQ ID NO 4;

a glycine at position 490 of SEQ ID NO 4; and

a glutamic acid at position 496 of SEQ ID NO 4;

using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™ ,available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

intron G	1.3kb	5845 C→T 6911 T→C		2/40 33/50
exon 8	136bp	835 G→A 853 G→A	arg270his arg275his	16/52 1/54
intron H				
exon 9	91bp			
intron I	1.7kb			
exon 10	64bp			
intron J	84bp			
exon 11	149bp	1068 G→A 1096 C→G	ala348tyr thr357ser	18/62 5/66
intron K				
exon 12	101bp	1315 C→G	pro430arg, splice site	4/66
intron L	3.8kb			
exon 13	497bp	1324 C→T 1405 A→G 1448 C→T 1494 A→G 1513 A→C 1628 G→T 1772 G→A	ala433val gln460arg silent ser490gly glu496ala silent silent	1/54 3/54 2/54 2/54 8/54 2/52 24/54

Positions in the 5' UTR refer to SEQ ID NO: 1.

Positions in exons refer to SEQ ID NO: 2.

Positions in introns refer to SEQ ID NO: 3.

Positions in protein refer to SEQ ID NO: 4.

exon 11	1068 A 1096 G
exon 12	1315 G
exon 13	1324 T 1405 G 1448 T 1494 G 1513 C 1628 T 1772 A

Region	Variant SEQ ID NO: 3
intron E	4780 T 4845 T 4849 C
intron F	5021 C 5554 $(GTTT)_n$, n=4 5579 C 5535 T
intron G	5845 T 6911 C

4 A nucleotide primer which can detect a polymorphism as defined in claim 1.

5 An allele specific primer capable of detecting a $P2X_7$ gene polymorphism as defined in claim 1.

6 An allele-specific oligonucleotide probe capable of detecting a $P2X_7$ gene polymorphism as defined in claim 1.

7 Use of a $P2X_7$ gene polymorphism as defined in claim 1 as a genetic marker in a linkage study.

8 A method of treating a human in need of treatment with a drug acting at $P2X_7$ in which the method comprises:

i) diagnosis of a polymorphism in $P2X_7$ in the human, which diagnosis preferably comprises determining the sequence at one or more of the following positions: positions 936, 1012, 1147, 1343 and 1476 in the 5'UTR region of the $P2X_7$ gene as defined by the position in SEQ ID NO: 1;

ABSTRACT

TITLE:CHEMICAL COMPOUNDS

This invention relates to polymorphisms in the human $P2X_7$ gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the $P2X_7$ gene, and to the use of $P2X_7$ polymorphism in treatment of diseases with $P2X_7$ drugs.

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